DNA Repair

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Complex DNA repair mechanisms evolved in cells to protect the integrity of genetic information from exogenous and endogenous damage. Distinct restoring pathways emerged for base and nucleotide excision, mismatch by insertion, deletion or base substitution, recombination repair, and translesion synthesis.[1] With better understanding of these molecular processes, opportunities come out that therapeutically exploit the differences between tumor and normal cells in their capacity to restore DNA damage.

SUMMARY OF PRESENTATIONS

DNA Repair Biomarkers

Dr. George R. Simon used the example of the excision repair cross-complementation 1 gene (*ERCC1*) as a valid predictive marker in clinical research. The product of the *ERCC1* gene is a key enzyme in nucleotide excision repair, which eliminates platinum DNA adducts.[1] When used as a biomarker, *ERCC1* was shown to predict for platinum sensitivity. Several clinical studies were presented with *ERCC1* as a selective biomarker: Adjuvant Trial IFCT0704 in nonsquamous stages II and IIIA where in the EGFR/wild-type arm *ERCC1* presence selected patients to observation, whereas its absence led to cisplatin pemetrexed therapy. Another trial to be conducted by the Southwest Oncology Group is a pilot proof of concept study with pharmacogenomic-directed adjuvant therapy in stage I NSCLC. Besides ERCC1, ribonulceotide reductase M1 (RRM1) was used in an Automated Quantitative Analysis (AQUA)-based assay. *RRM1* encodes two subunits of ribonucleoside-diphosphate reductase, an enzyme essential for the production of deoxyribonucleotides before DNA synthesis in S phase of dividing cells and predicts for sensitivity to gemcitabine.[2] The MADeIT trial stands for a phase II study on the use of molecular analyses-based customized chemotherapy in patients with stage IV/IIIB (malignant pleural effusion) NSCLC. With 59% 12-month overall survival, the study suggests that ERCC1 and RRM1-based molecular analysis applied in clinical setting has survival benefits superior to previous studies with simple combinations of agents used here (docetaxel, navelbine, gemcitabine, and carboplatin, Figure 1).

A phase III study is under way with the MADeIT algorithm built-in in one arm compared with standard doublet of carboplatin and gemcitabine. In early-stage disease, high ERCC1 identifies a subset of patients who are less likely to relapse and derive no additional benefit from platinum-based chemotherapy. Low ERCC1 on the other hand identifies a subset with poor prognosis but with benefit from platinum-based treatment.

Another well-studied repair marker is the tumor suppressor BRCA1 (Breast Cancer Type 1 gene/protein). Mutations of *BRCA1* and *2* strongly predispose individuals to cancer because they cause a deficiency in DNA repair through homologous recombinantion. As a predictive biomarker, *BRCA1* may, besides sensitivity to platinum, also identify a cohort of patients that respond to docetaxel.

Poly(Adenosine Diphosphate-Ribose) Polymerase Inhibitors

Poly(adenosine diphosphate-ribose) polymerase (PARP) facilitates base excision repair.[3] Inhibition of members of the PARP family can selectively kill *BRCA1* and *BRCA2*-deficient cells. The benzimidazole derivative ABT-888 is a potent inhibitor of PARP1 and 2. In his presentation, Dr. Suresh Ramalingam reported that in preclinical models, ABT-888 potentiated the effect of temozolomide, cisplatin, cyclophosphamide, irinothecan, and radiation therapy. An exploratory phase 0 study with ABT-888 established the target assay feasibility with no treatment-related adverse events reported so far. The bioavailability of the drug is >50% with oral administration. ABT-888 has demonstrated tolerability as a single agent and with other drugs in early-phase clinical studies. Its combinations with carboplatin and paclitaxel are feasible, and a phase II study in advanced NSCLC is under development.

Another PARP inhibitor, AG-014699, is a small molecule IV agent that has demonstrated activity against PARP by potentiating antitumor effects of radiation in preclinical models, by enhancement of drugs that break DNA strands (e.g., temozolomide and topoisomerase inhibitors) and by inhibiting PARP enzyme activity in peripheral blood in a PD phase 1 trial. Dr. Mace Rothenberg from Pfizer reported that AG-014699 has shown promising activity as a cytotoxic potentiating agent when used in conjunction with temozolamide in patients with metastatic melanoma and as a single agent in women with *BRCA*-positive breast and ovarian cancer. At present, AG 014699 is being examined in a phase II proof of principle IIR study in known carriers of a *BRCA1* or *BRCA2* mutation with metastatic breast or ovarian cancer. About 30% of NSCLC carry a *BRCA* mutation, and this loss of function is associated with a worse prognosis in patients with resected disease. AG-014699 is being considered for a

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phase II trial in patients with metastatic NSCLC with *BRCA* mutation. Considerations whether *BRCA* status alone can stand as an independent prediction test are still ongoing.

Aurora Kinase A Inhibitor

Aurora kinases A, B, and C are involved in cell cycle regulation through centrosome function, bipolar spindle assembly, and chromosomal segregation process.[4] In his review, Dr. David Mauro from Merck reported that Aurora A kinase is required for entry into a progression through mitosis, and it can induce oncogenic transformation. Overexpression of Aurora A was detected in breast, lung, pancreatic, bladder, esophageal, ovarian, renal, head and neck cancers. It is amplified in breast, colon, bladder, head and neck cancers. Aurora A can be suppressed by RNAi that leads to tumor growth suppression and enhanced taxane sensitivity. Known functional interactions between Aurora A and p53 and cell-line data suggest greatest efficacy will be in p53 context. Aurora A is a preclinically validated target for therapeutic intervention as its inhibition in combination with taxanes produces robust synergy over taxanes alone in multiple cell lines and xenograft models. Efficacy in monotherapy was also demonstrated in some xenograft models. An inhibitor MK-5108 was successfully used in preclinical cell line and animal models together with predictive biomarkers pHH3 and qPCR signature. Aurora A kinase and p53 interact at multiple levels. Preclinical data with MK-5108 and other Aurora A inhibitors suggest p53-negative tumors may be more sensitive to Aurora A inhibitors than p53-positive tumors. A phase I, open label, randomized, two panel (monotherapy and combination) dose escalation trial of MK-5108 alone or with docetaxel in adult patients with locally advanced or metastatic solid tumors was reported. Study endpoints include safety, PK/PD, and maximum tolerated dose relationships, and objective tumor response in monotherapy only.

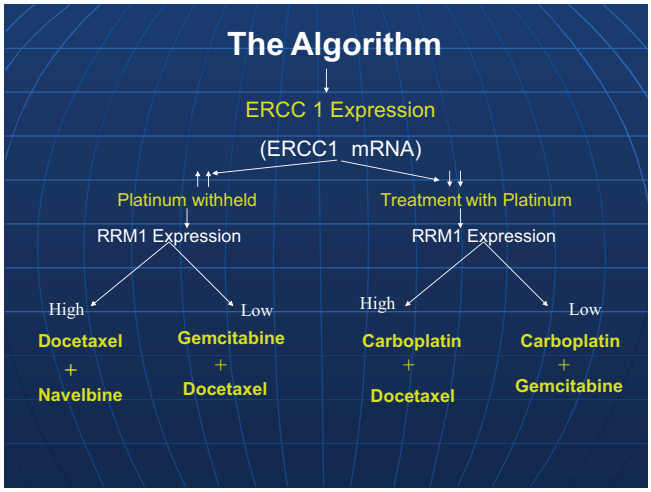

FIGURE 1. MADeIT trial concept (Simon, printed with permission).

WEE1 Inhibitor

The protein WEE1 catalyzes inhibitory tyrosine phosphorylation of CDC2/cyclin B kinase and seems to coordinate the transition between DNA replication and mitosis by protecting the nucleus from cytoplasmically activated CDC2 kinase.[5] Preclinical data indicate that inhibition of WEE1 may abrogate G2 checkpoint arrest in the selective sensitization of certain tumor cells. A new WEE1 inhibitor MK-1775 was reported by Dr. Mauro as a p53 context-specific DNA damage sensitizer. It specifically sensitizes p53-negative cells to DNA damage by abrogation of the G2 checkpoint as shown in in vitro studies with gemcitabine, carboplatinum, and doxorubicin. Encouraged by preclinical studies, a three-arm phase I trial was designed with the use of gemcitabine, cisplatin, or carboplatin in combination with MK-1775 in patients with p53-deficient tumors. The study is almost completed, and phase II studies will commence

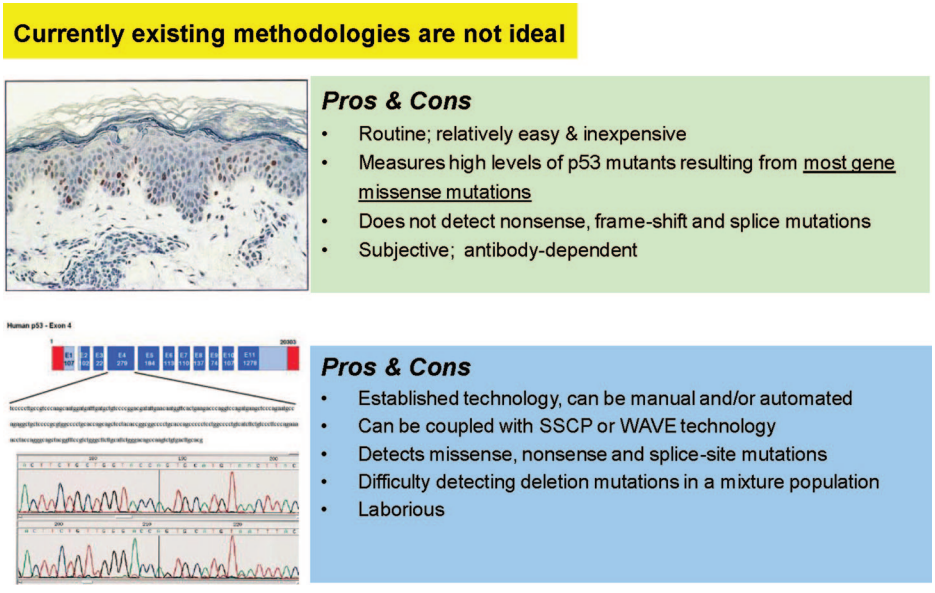

FIGURE 2. Current technologies for detecting of *p53* mutation: Immunohistochemistry vs. DNA sequencing analysis. (Mauro, printed with permission).

in the second half of 2009. Of question remains the choice of assays measuring p53 because available tests have still to reach desirable requirements.

Glutathione Disulfide-Mimetic

Even though this agent is not involved in DNA repair, it is discussed here briefly. Dr. Thomas Lynch introduced a novel redox modulating agent NO-002. The drug alters cellular redox status and activity of redox-sensitive proteins, resulting in pleiotropic effects on cell functions such as activation of signaling pathways, cytoskeletal changes, and altered cytokine production. A Russian phase II study in chemonaive advanced NSCLC showed 63% 1-year survival in the NOV-002 plus cisplatin versus 17% in cisplatin alone. A significant increase in tolerance to chemotherapy with 66% more cycles received was observed in the combination arm. A US phase I/II trial in patients with advanced NSCLC used paclitaxel and carboplatin with or without NOV-002. Objective tumor response was seen in 69% of patients with NOV-002 against 33% in the chemotherapy alone arm. A pivotal phase III study in advanced NSCLC is being conducted in an international consortium with overall survival as the primary efficacy endpoint. It is an open label randomized trial of carboplatinum/paclitaxel ± NOV-002. Standard doses of carboplatinum/paclitaxel are combined with NOV-002 and given IV on day 1 then SC on day 1 to 21. The event-driven final analysis is expected by the end of 2009. In other cancers, a completed phase II US study in platinum-resistant ovarian cancer was reported where the median progression-free survival lasted 15.4 weeks (nearly 2× historical control). A clinical benefit was observed in 60% of evaluable patients. There is an ongoing phase II neoadjuvant trial in breast cancer in combination with chemotherapy, also in the United States.

SUMMARY

Information about the molecular processes associated with DNA repair has led to the development of pharmacogenomic biomarkers for customized therapy on one hand and to the design of small molecules that target DNA repair mechanisms on the other. Both areas are rapidly evolving and produce promising leads. A number of phase II and phase III clinical trials are under way that may move the field forward. There is still a need to improve existing biomarker assays for their optimal accuracy and practicality of use. Examples are tests for p53 or ERCC1 where immunochemistry, mutation analysis, and/or reverse-transcriptase polymerase chain reaction show mutually exclusive advantages and drawbacks (Figure 2).

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